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Report

Experimental Evolution of Mating Discrimination in Budding Yeast

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Summary

Assortative mating, when individuals of similar phenotypes mate, likely plays a key role in preventing gene flow during speciation. Reinforcement occurs when two previously geographically separated (allopatric) groups meet after having evolved partial postzygotic isolation; they are selected to evolve or enhance assortative mating to prevent costly intergroup matings that produce only maladaptive or sterile hybrids [1–4]. Studies in *Drosophila* have shown that the genetic architectures of mating discrimination could differ significantly with or without reinforcement [5], suggesting that the evolution of assortative mating may be more complicated than expected. To study the evolution of assortative mating, we evolved mating discrimination in populations of the budding yeast, *Saccharomyces cerevisiae*. After 36 cycles of selection, these cells are five times more likely to mate with each other than to their ancestors, despite detectable one-way gene flow between the selected and reference populations. Several individual cultures evolved mating discrimination by changing their mating kinetics, with some mating more rapidly and others more slowly than the ancestral population. Genetic analysis indicates that multiple mutations have accumulated to produce the altered mating preference. Our results show that subtle details of mating behavior can play an important role in the evolution of reproductive isolation.

Results and Discussion

Haploid yeast cells exist as one of two mating types, **a** and α, which are specified by two alternative alleles at the mating-type locus (*MAT*). They can be propagated asexually as either haploids or diploids, mating only occurs between opposite mating types, and the major signal-transduction pathways involved in mating have been identified [6]. Three features ensure that mutations that produce mating discrimination appear during the experimental selection. First, genetic markers can be introduced by transformation so that two different strains can be easily derived from a common background, without the introduction of uncontrolled genetic variation. Second, mutation rates can be elevated by removal of the mismatch repair gene *MSH2*; removal of this gene increases the mutation rate about 100-fold [7] over values (roughly 10^{-9} per bp per generation mutation) reported for laboratory strains [8]. Third, large population sizes are easily maintained during selection, making it possible to select rare mutations.

The basic selection for altered mating preference applies selective pressure for cells in an evolving (E) population to mate with each other rather than with a reference (R) population (Figure 1A). We derived the two strains from the same ancestor by adding several nutrient-requirement and drug-resistance markers (see Experimental Procedures). The evolving strain, which lacked Msh2, was passed through multiple rounds of selection, whereas the reference strain was revived from a frozen stock for each round of selection. In each round of selection, about 2×10^6 evolving cells (E cells) were mixed with the reference cells (R cells) in a 1E:10R ratio and allowed to mate. Initially, E cells cannot discriminate between themselves and their more numerous R neighbors, and only 10% of the E cells will mate with another E cell. A dominant suicide gene in the R strain was induced after mating, killing more than 95% of E × R hybrids and R × R diploids. The preferential survival of E × E diploid cells created a strong selection for E population mutants that prefer mating to other E cells but did not completely prevent gene flow from the R population; in the initial cycles, 2.3 ± 0.5% of the selected progeny expressed genetic markers derived from the R population, demonstrating limited gene flow from the R strain to the E population. The surviving diploid cells were then propagated and induced to sporulate, and the **a** and α haploids were separately recovered with drug-resistance and nutrient-requirement markers linked to the mating-type locus and used to seed the next round of selection. The dominant suicide gene in the reference strain is a cohesin-subunit (*MCD1*) nondegradable mutant placed under the control of the inducible *GAL1* promoter. Inducing the mutant gene kills most of the cells that contain it by disrupting chromosome segregation [9] and thus greatly reducing the fitness of the hybrids between E and R strains. During each round of selection, the population goes through a bottleneck of about 10^5 cells. To test whether there are one or many evolutionary paths to an altered mating preference, our experiment began with 40 individual cultures, of which 13 survived to the end of the experiment. The rest were abandoned because of contamination by bacteria or other fungi (5 out of 27) or mutations that compromised the markers used for selection (22 out of 27). The high mutation rate in the selective markers is probably due to the use of a mutator as our evolving strain.

After 36 rounds of selection, 11 out of 13 evolved populations significantly increased their preference for mating within their own population (Table 1). Thus, strong selection can rapidly change the mating preference of a population. In addition, different evolved cultures show various levels of mating preference, suggesting that they have different evolutionary dynamics, have

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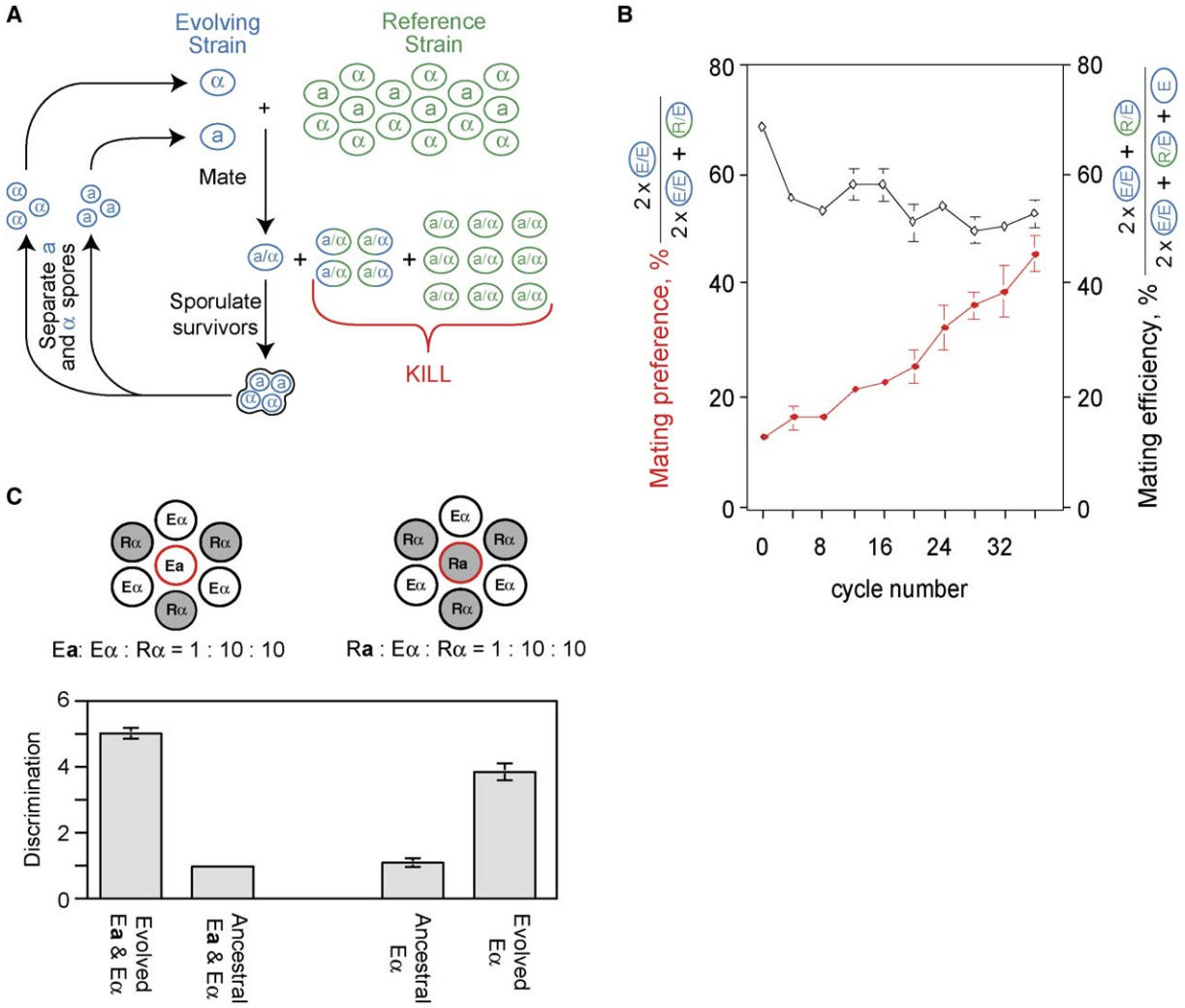


Figure 1. Evolution of Assortative Mating under Laboratory Selection

(A) Experimental design of selection for new mating preference.

(B) Evolution of mating preference and mating efficiency of the culture E42. E haploid cells are mixed with a 10-fold excess of R haploid cells in the mating assay. Mated diploid cells and unmated haploid cells are counted after 5 hr of mating at 30ºC.

Mating preference = (2(E × E diploids))/(E × R diploids + 2(E × E diploids))

Mating efficiency = (E × R diploids + 2(E × E diploids))/(E haploids + E × R diploids + 2(E × E diploids))

(C) Experimental design and results of the discrimination assay. **a** and α cells are mixed in a 1:20 ratio and allowed to mate at 30ºC for 5 hr. Evolved **Ea** and Eα are the haploid cells of the culture E42 at cycle 36. Discrimination = (number of Ea × Eα diploids)/(number of Ea × Rα diploids), when Ea is the minority, or (number of Ra × Rα diploids)/(number of Ra × Eα diploids), when Ra is the minority.

Error bars are ± 1 standard deviation.

followed different genetic paths, or both. In Figure 1B, we show the intra-population mating preference (the fraction of mated E cells that mate with each other, as opposed to R cells) and total mating efficiency (the fraction of E cells that mate to any partner) of one culture (E42) over the selection cycles. It shows that the mating preference has increased gradually but that the mating efficiency was only slightly affected. There is a modest negative correlation between mating preference and mating efficiency across the complete set of evolved cultures (correlation coefficient = −0.62, p = 0.025). For culture E43 we measured the gene flow between evolved and reference populations at the end of the experiment. After 36 cycles, only 0.04 ± 0.01% of the selected progeny had markers derived from the R strain, indicating that populations that had evolved a preference for intra-population mating had strongly decreased the level of genetic exchange with the reference population.

If cells have genuinely changed their mating preference, the evolved and reference strains should mutually discriminate against each other. We showed this by using a discrimination assay, in which **a** cells are mixed with a large excess of α cells, half from the E strain and half from the R strain, as shown schematically in Figure 1C. After 36 cycles, the evolved **Ea** cells show a 5-fold bias toward mating with Eα cells as opposed to Rα cells. To eliminate the possibility that evolving cultures had just become "sexier," we asked if reference cells discriminated against them when given a choice

Table 1. Mating Preference and Mating Efficiency of the Evolved Cultures after 36 Cycles of Selection

Evolved Line	Mating Preference	Mating Efficiency
E12	49 ± 3%[a]	40 ± 4%[a]
E14	48 ± 2%[a]	52 ± 2%[a]
E42	45 ± 3%[a]	53 ± 3%[a]
E5	44 ± 3%[a]	37 ± 3%[a]
E3	39 ± 3%[a]	56 ± 2%[a]
E2	39 ± 2%[a]	37 ± 3%[a]
E41	38 ± 1%[a]	51 ± 3%[a]
E15	38 ± 3%[a]	53 ± 4%
E4	37 ± 3%[a]	54 ± 2%[a]
E13	36 ± 3%[a]	60 ± 4%
E43	36 ± 2%[a]	52 ± 3%[a]
E17	22 ± 3%	56 ± 1%[a]
E1	21 ± 3%	65 ± 5%
Ancestral	14 ± 1%	66 ± 1%

Mating preference and efficiency were measured as described in the legend to Figure 1. The 14% mating preference (as opposed to the expected 10%) in the ancestor is due to the initial frequencies of the mating type, not due to any true preference.

[a] The observed value in the evolved strain is significantly different ($p < 0.05$, two-tailed t test) from the value of the ancestral strain.

of partners. The **a** cells from the R strain have about a 4-fold bias against mating with the evolved E cells, showing that a new mating preference has evolved. We saw a similar bias in reciprocal experiments, in which α cells are mixed with a large excess of **a** cells and mating discrimination of the α cells is measured (data not shown), indicating that the mating discrimination we have evolved is not mating-type specific.

To examine the population structure of the evolved cultures, we isolated about 30 single colonies from each individual culture and measured their mating preference. The results indicate that all the cultures are composed of heterogeneous populations (data not shown). From the cultures E2, E5, E42, and E43, a single **a** and a single α clone that had the highest mating preference were used for further phenotypic characterization.

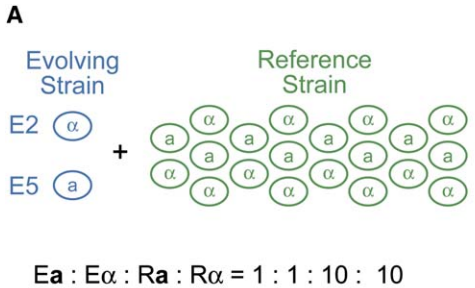
In principle, mating preferences in different cultures could have evolved as a result of the alteration of different aspects of mating behavior. We sought to discover whether different evolved cultures had evolved compatible phenotypes by mixing **a** cells from one evolved culture and α cells from another with an excess of R cells and measuring their mating preference (Figure 2A). If different cultures have evolved incompatible phenotypes, the mating preference measured between **a** and α of different cultures should be lower than the average of the two intrapopulation mating preferences. Crosses that involved the culture E2 had substantially lower mating preferences than those of the intrapopulation matings (Figure 2B). In all the other combinations, mating between strains derived from different cultures was as favorable as that between strains from the same culture. These results suggest that three of the cultures have evolved compatible phenotypes. The simplest explanation of this observation is that the phenotype in these three cultures is physiologically similar. We can rule out the possibility that different cultures arose by a single event followed by cross-contamination because different mutations were observed at the same locus in different cultures (our unpublished data).

Genetic analysis shows that the selected phenotypes are caused by multiple mutations. We crossed evolved cells from three different cultures (E5, E42, and E43) to the ancestral strain and examined the phenotype of the haploid progeny. In all three crosses, less than 10% of these cells had a mating preference similar to that of their evolved parent, and most of the cells had mating preferences that were intermediate between the evolved and ancestral values, and the results from one cross are shown in Figure 2C. If the evolved phenotype is caused by *N* unlinked mutations with additive effects, the proportion of progeny with the same mating preference as their evolved parent should be $(1/2)^N$. Therefore, at least three mutations must have accumulated to produce the altered mating preference. The distribution of mating preferences also gives information about the interactions among the mutations. If each mutation acts additively and independently, the distribution of mating preferences should be symmetric (e.g., in the simplest case, when all mutations act equally, the probability of segregants with n mutations is $(1/2)^n \times N!/n!(N-n)!$, which will give a symmetric distribution because $N!/n!(N-n)!$ is a symmetric function. In a more complicated case, when mutations have different effects, the symmetry still holds.). The observed skew toward weaker mating preference in Figure 2C suggests that some mutations only have a phenotypic effect when other mutations are present (epistasis).

Do populations with similar phenotypes have mutations in the same genes? Because the clones from the cultures E42 and E43 have very similar phenotypes, we asked whether they share the same spectrum of mutations. Haploid progeny isolated from either the cross between these two cultures or the cross within the same culture were measured for the mating preference. As shown in Figure 2D, a broader distribution of individual mating preference was observed for crosses between the hybrid progeny ($p < 0.01$, one-tailed F test). It indicates that the E42 and E43 clones do not have exactly the same mutations even though their phenotypes are very similar to each other. These differences could reflect mutations of different strengths within the same genes or genes that have been mutated in only one of the two cultures.

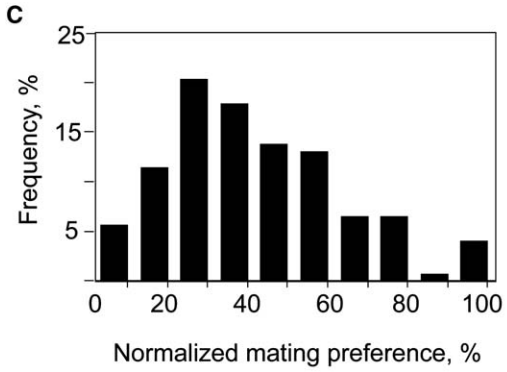
Hartwell and his colleagues showed that yeast cells communicate as they mate with each other [10]. If this communication changes as two cells "court" each other, cells that respond with similar kinetics are most likely to mate with each other. Thus, one way of changing mating preference might be to alter mating kinetics. We measured the kinetics of mating by mixing **a** cells with a 10-fold excess of α cells from the same culture and determining the fraction of **a** cells that had mated at different times. The results show that all the evolved cells have changed their mating kinetics (Figure 3A). All the evolved cells except the E2 cells mate more rapidly than ancestral cells. These kinetic observations match the results of matings between different evolved cultures (Figure 2B). In both assays, E2 differs from the rest of the evolved cultures in that it mates more slowly than the ancestors and mates poorly to the evolved clones from other cultures.

Do altered mating kinetics contribute to altered mating preference? We tested whether these two



B

	E2α	E5α	E42α	E43α
E2a	38 ± 2	23 ± 2**	23 ± 3**	23 ± 3**
E5a	26 ± 4**	46 ± 2	46 ± 5	46 ± 4
E42a	26 ± 1**	44 ± 2	46 ± 2	46 ± 4
E43a	22 ± 7	48 ± 4	44 ± 2	48 ± 3



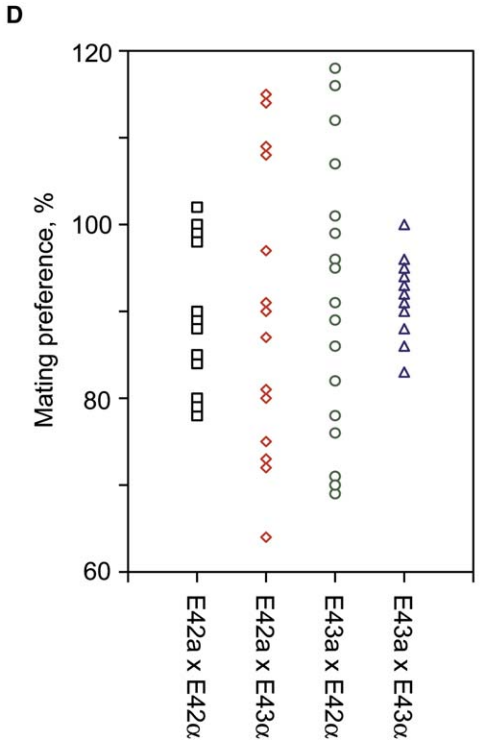


Figure 2. Mating Discriminations between Different Evolved Cultures

(A) Schematic diagram showing mating between two different evolved cultures. One **a** and one α clone from two different evolved cultures were mixed with a 10-fold excess of R haploid cells in the mating assay.

(B) Two incompatible routes to altered mating preference. Clones from different cultures were mated to each other as shown in (A). The ancestral level of mating preference is ~10%. **, the mating preference measured between **a** and α of different cultures is significantly different ($p < 0.05$, two-tailed t test) from the average of the two values obtained when **a** and α are from the same culture.

(C) Mating preference of the F1 progeny from the cross between the ancestral strain and the evolved culture E43. One **a** and one α clone were isolated from the culture E43 at cycle 36. The α clone was mated to the ancestral clone and sporulated to produce the F1 haploid progeny. The **a** clone was used as the mating partner in the mating assays. A total of 125 F1 α clones was isolated and measured for mating preference. The individual mating preferences are normalized to the mating preference observed in the mating between the E43**a** and E43α clones.

(D) The distribution of mating preferences of individual clones. Single **a** and α clones isolated from the cultures E42 and E43 at cycle 36 were mated to each other and sporulated. In each cross, we measured the mating preference of 18 **a** haploid progeny. The original E42α clone was used as the mating partner in the mating assays for the clones from the crosses E42**a** × E42α and E42**a** × E43α, and the original E43α clone was used as the mating partner in the assays for the clones from the crosses E43**a** × E42α and E43**a** × E43α. All the individual mating preferences are normalized to the mating preference seen in the mating between the original E43**a** and E43α clones. Means and standard deviations of the mating preferences measured from all the clones are: E42a × E42α = 87.6 ± 7.7%; E42a × E43α = 90.3 ± 15.9%; E43a × E42α = 91.8 ± 15.6%; and E43a × E43α = 92.3 ± 4.0%.

phenotypes co-segregate in a cross between evolved and ancestral cells. In 66 F1 haploid **a** clones isolated from the cross between E43 and ancestral cells, four have a high mating preference (close to that of the evolved parent), and five have a low mating preference (close to that of the wild-type parent). The mating preferences of these nine clones are correlated with their mating speeds ($p < 0.002$, Figure 3B). When 21 clones that have intermediate mating preferences are included, the significance of the correlation between mating preference and mating speed becomes even greater (correlation coefficient = 0.86, $p = 1.8 \times 10^{-9}$), indicating that the altered mating kinetics in the evolved cells contributes to their evolved mating preference.

These kinetic differences could affect the timing of two processes, the initial response of cells to their neighbors' pheromones or their communication and commitment to each other after their initial detection

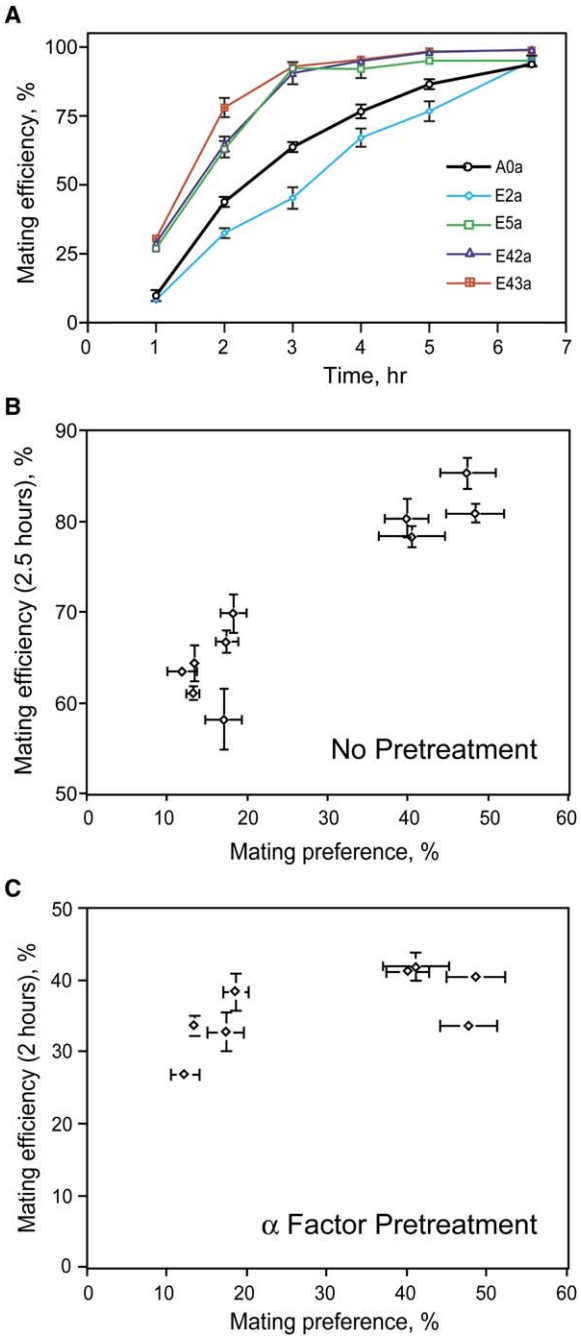


Figure 3. Change in Mating Speeds Results in Mating Discrimination

(A) Evolved strains have different mating kinetics. **a** and α cells from the same evolved cultures were mixed in a 1:10 ratio to mate at 30°C, and the frequency of the **a** cells that had formed diploids was measured at the indicated times. Each sample point is the average of three measurements, and the standard deviation is presented. The mating efficiencies shown in this experiment are noticeably higher than the mating efficiencies shown in Table 1 or Figure 1B because a 10-fold excess of α cells was used in this experiment and only the mating efficiencies of **a** cells are shown here.

(B) Mating speed is correlated with mating preference in segregants of a cross between the evolved culture E43 and the ancestral strains. The mating preference of **a** cells from this cross was measured as previously described. Mating efficiency was measured 2.5 hr after cells were mixed. The same E43 α clone was used as the mating partner for all measurements. To rule out the possibility that the low-mating -reference group has general mating defects, we also measured the mating efficiency at 6 hr. By this time, both groups had approached 100% mating efficiency.

(C) Pre-activating the mating pathway can reduce the difference in mating speeds between the high-mating-preference and the low-mating-preference groups. **a** cells were pre-treated with 10 μg/ml α-factor for 1 hr before being mixed with α cells. Mating was conducted on α-factor-containing plates (1 μg/ml). Mating efficiency was measured 2 hr after strains were mixed.

Error bars are ± 1 standard deviation.

of pheromone. We distinguished these possibilities by pre-treating the **a** segregants from the ancestral × evolved cross with α factor before exposing them to α cells. If the more discriminating segregants detect pheromone more quickly, pre-activating the pheromone signaling pathway in **a** cells should eliminate their kinetic advantage over the less-discriminating segregants. Figure 3C verifies this prediction by showing that the rapidly mating and slowly mating segregants exhibit similar mating speeds when we pretreated the **a** cells with α factor and then mated the cells on plates containing a low concentration of α factor. To examine whether the evolved strains have altered their pheromones or pheromone receptors, we sequenced the genes encoding the pheromones (*MFA1, MFA2, MFalpha1, MFalpha2*) and pheromone receptors (*STE2, STE3*) in the evolved E43 clones. No mutation was observed in the ORFs or 300-bp-upstream regions of these genes. This result is not surprising because it would require mutations in both a pheromone and its cognate receptor to produce mating discrimination, although the result does contrast with the existence of mutations in gamete receptors in several pairs of closely related species [11–13]. It is likely that mutations in receptors and pheromones only occur in the later stages of prezygotic isolation when populations are already partially separated as a result of mutations that could act alone rather than requiring a complementary mutation in an interacting gene.

In previous studies of *Drosophila* and maize, "destroy-the-hybrids" selections have successfully evolved mating discrimination between two genetically marked populations [14–17]. Unfortunately, these experiments are constrained by small population sizes, the amount of genetic variation both within and between the starting populations is uncertain, and the detailed genetic basis of the evolved phenotypes is not known. In many of these experiments, gene flow between the two populations has been deliberately prevented. In those where gene flow occurred, the vast majority failed to evolve any mating preference. The only exception, a study by Thoday and Gibson [18], could not be repeated. Previous studies of experimental evolution in the budding yeast have helped dissect the mechanism of adaptation to metabolic challenges and antifungal drug treatments [19, 20]. Here, we show that mating preference, a complicated phenotype involving gradient sensing and complicated communication between cells, can be evolved in the laboratory. Multiple cultures have evolved new mating preferences by changing their mating kinetics, indicating that changing mating kinetics may be a useful strategy for preventing mating between different populations. A further analysis of the evolved phenotype to identify the mutations responsible for the altered mating

preference will help us understand the molecular and cellular bases of adaptation.

Experimental Procedures

Strains and Genetic Procedures

Yeast strain genotypes are listed in Table S1, available with this article online. Evolving strains (JYL243 and JYL246) and reference strains (JYL209 and JYL210) are isogenic with *W303* (*MAT**a** ura3-1 his3-11,15 leu2-3,112 trp1-1 ade2-1 can1-100*). Substitutive and integrative transformations were carried out by the lithium acetate procedure [21]. Media and microbial and genetic techniques were as described [22].

Selection Cycles

Evolving cells (**a** and α; 2×10^6) were mixed with reference cells (R**a** and Rα; 2×10^7) and then spread on a 5 cm^2 area of YPD plates. Cell numbers were estimated from the optical densities of the cell cultures with a spectrophotometer (Beckman Coulter, DU640B). After 6 hr of mating at 30ºC, cells were washed off the plates and diluted into 12 ml of complete synthetic medium (CSM)-Ura-Trp-Lys + Galactose, which selects for diploid formation and induces *pGAL1-MCD1-DD* [9], the suicide gene carried by the reference strain. Cultures were grown for 48 hr, a treatment that kills more than 95% of E × R hybrid cells and R cells. During this period, selected diploid cells grow about 10 generations. Three hundred microliters of these saturated cultures were then diluted into 3 ml of YPA (yeast extract, peptone, potassium acetate) medium and grown for 20 hr before being transferred into sporulation medium for 48 hr. Sporulated cells were digested with 80 μg/ml zymolyase (Seikagaku America, St. Petersburg, FL) for 1 hr, washed with 1% SDS solution, and then sonicated for 2 min. The digested spores were split into two parts to select separately for **a** and α spores. The basis of the selection is complementary drug-resistance and nutrient-independence markers that are linked to the mating-type locus. In **a** cells, the *MAT* locus is flanked by *LYS5*, which allows growth in the absence of lysine but confers sensitivity to α-aminoadipate [23] and *FUR1*, which confers sensitivity to 5-fluorouracil [24]. In α cells, the *MAT* locus is flanked by *URA3*, which allows growth in the absence of uracil but confers sensitivity to 5-fluoroorotic acid [25], and *TRP5*, which allows growth in the absence of tryptophan but confers sensitivity to 5-fluoroindole [26]. One portion of the spores was inoculated into CSM-Lys + 1 mg/ml 5-fluoroorotic acid (US Biological, Swampscott, MA) + 0.015% 5-fluoroindole (Sigma-Aldrich, St. Louis, MO) to select for E**a** cells. The other was inoculated into minimal medium + 2 mg/ml α-aminoadapate (Sigma-Aldrich, St. Louis, MO) + 0.4 mg/ml 5-fluorouracil (Sigma-Aldrich, St. Louis, MO) to select for Eα cells. E**a** and Eα cells were then grown in rich medium before the mating that marked the start of the next round of selection. During the whole procedure, the effective population size was maintained above 10^5. There were about 20 cell generations between each round of mating.

Mating Assays and Discrimination Assays

In the mating assay, 2×10^6 evolving cells (E**a** and Eα) were mixed with 2×10^7 reference cells (R**a** and Rα) and then spread on a 5 cm^2 area of YPD plates. Cell numbers were estimated from the optical densities of the cell cultures with a spectrophotometer (Beckman Coulter, DU640B). After 5 hr of mating at 30ºC, cells were washed off and plated on CSM-Ade plates at a density of about 300 colonies/plate. The number of various cell types was determined by replica plating these colonies onto different selective plates. In the discrimination assay, 10^6 **a** cells were mixed with 2×10^7 α cells and then spread on a 5 cm^2 area of YPD plates. After 5 hr of mating at 30ºC, cells were plated on CSM-Lys plates, and the number of different cells type was determined as described in the mating assay. In both assays, at least three independent mating plates were set up, and their average is shown at each sample point.

Supplemental Data

Table S1 is available with this article online at http://current-biology.com/cgi/content/full/16/3/280/DC1/.

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References

1. Butlin, R. (1987). Speciation by reinforcement. Trends Ecol. Evol. *2*, 8–13.
2. Noor, M.A. (1999). Reinforcement and other consequences of sympatry. Heredity *83*, 503–508.
3. Lukhtanov, V.A., Kandul, N.P., Plotkin, J.B., Dantchenko, A.V., Haig, D., and Pierce, N.E. (2005). Reinforcement of pre-zygotic isolation and karyotype evolution in Agrodiaetus butterflies. Nature *436*, 385–389.
4. Hoskin, C.J., Higgie, M., McDonald, K.R., and Moritz, C. (2005). Reinforcement drives rapid allopatric speciation. Nature *437*, 1353–1356.
5. Ortiz-Barrientos, D., Counterman, B.A., and Noor, M.A. (2004). The genetics of speciation by reinforcement. PLoS Biol. *2*, e416. 10.1371/journal.pbio.0020416.
6. Elion, E.A. (2000). Pheromone response, mating and cell biology. Curr. Opin. Microbiol. *3*, 573–581.
7. Reenan, R.A., and Kolodner, R.D. (1992). Characterization of insertion mutations in the Saccharomyces cerevisiae MSH1 and MSH2 genes: Evidence for separate mitochondrial and nuclear functions. Genetics *132*, 975–985.
8. Drake, J.W. (1991). A constant rate of spontaneous mutation in DNA-based microbes. Proc. Natl. Acad. Sci. USA *88*, 7160–7164.
9. Uhlmann, F., Lottspeich, F., and Nasmyth, K. (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature *400*, 37–42.
10. Jackson, C.L., and Hartwell, L.H. (1990). Courtship in S. cerevisiae: Both cell types choose mating partners by responding to the strongest pheromone signal. Cell *63*, 1039–1051.
11. Metz, E.C., and Palumbi, S.R. (1996). Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. Mol. Biol. Evol. *13*, 397–406.
12. Swanson, W.J., and Vacquier, V.D. (1997). The abalone egg vitelline envelope receptor for sperm lysin is a giant multivalent molecule. Proc. Natl. Acad. Sci. USA *94*, 6724–6729.
13. Swanson, W.J., and Vacquier, V.D. (2002). The rapid evolution of reproductive proteins. Nat. Rev. Genet. *3*, 137–144.
14. Koopman, K. (1950). Natural selection for reproductive isolation between Drosophila pseudoobscura and Drosophila persimilis. Evolution Int. J. Org. Evolution *4*, 135–148.
15. Paterniani, E. (1969). Selection for reproductive isolation between two populations of maize, Zea mays L. Evolution Int. J. Org. Evolution *23*, 534–547.
16. Crossley, S. (1973). Changes in mating behavior produced by selection for ethological isolation between ebony and vestigial mutants of Drosophila melanogaster. Evolution Int. J. Org. Evolution *28*, 631–647.
17. Dobzhansky, T., Pavlovsky, O., and Powell, J. (1976). Partially successful attempt to enhance reproductive isolation between semispecies of Drosophila paulistorum. Evolution Int. J. Org. Evolution *30*, 201–212.
18. Thoday, J.M., and Gibson, J.B. (1962). Isolation by disruptive selection. Nature *193*, 1164–1166.
19. Ferea, T.L., Botstein, D., Brown, P.O., and Rosenzweig, R.F. (1999). Systematic changes in gene expression patterns following adaptive evolution in yeast. Proc. Natl. Acad. Sci. USA *96*, 9721–9726.
20. Anderson, J.B., Sirjusingh, C., Parsons, A.B., Boone, C., Wickens, C., Cowen, L.E., and Kohn, L.M. (2003). Mode of selection

and experimental evolution of antifungal drug resistance in Saccharomyces cerevisiae. Genetics *163*, 1287–1298.

21. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*, 163–168.
22. Sherman, R., Fink, G., and Lawrence, C. (1974). Methods in Yeast Genetics (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).
23. Chattoo, B.B., Sherman, F., Azubalis, D.A., Fjellstedt, T.A., Mehnert, D., and Ogur, M. (1979). Selection of *lys2* mutants of the yeast *Saccharomyces cerevisiae* by the utilization of α-aminoadipate. Genetics *93*, 51–65.
24. Kern, L., de Montigny, J., Lacroute, F., and Jund, R. (1991). Regulation of the pyrimidine salvage pathway by the FUR1 gene product of Saccharomyces cerevisiae. Curr. Genet. *19*, 333–337.
25. Boeke, J.D., LaCroute, F., and Fink, G.R. (1984). A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. *197*, 345–346.
26. Toyn, J.H., Gunyuzlu, P.L., White, W.H., Thompson, L.A., and Hollis, G.F. (2000). A counterselection for the tryptophan pathway in yeast: 5-fluoroanthranilic acid resistance. Yeast *16*, 553–560.